

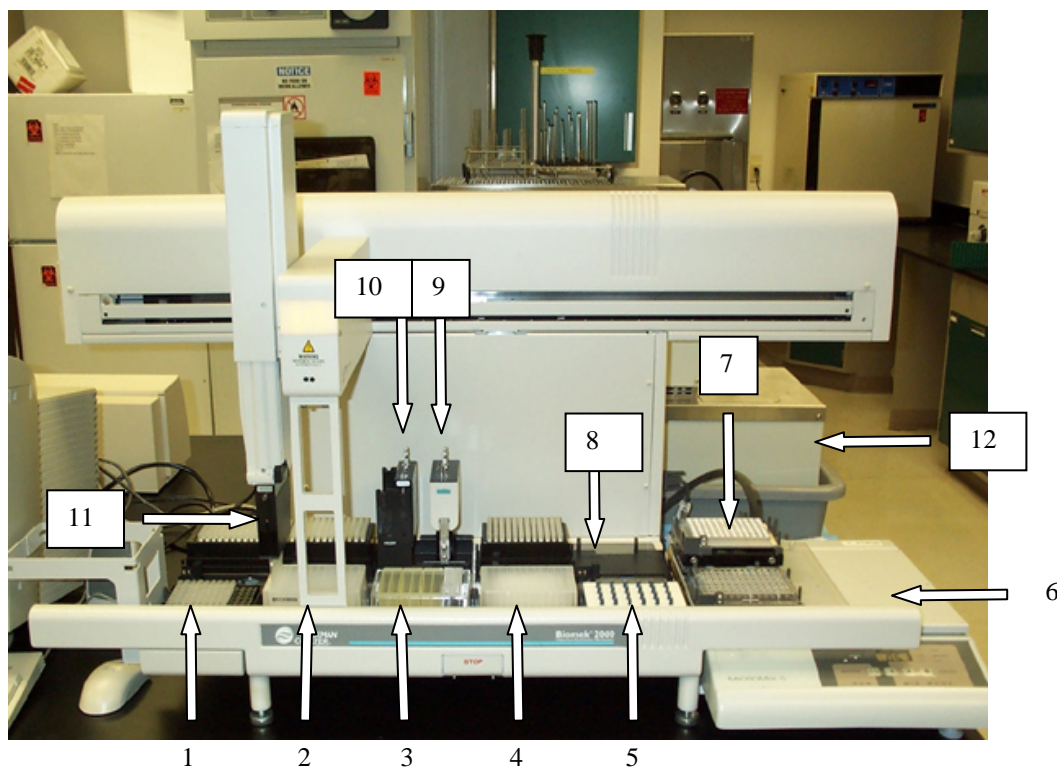
INTRODUCTION	Page v
BIOMEK®2000 AUTOMATION WORKSTATION PROCEDURES MANUAL - FORENSIC BIOLOGY SECTION PROCEDURE MANUAL, SECTION IV	Issue No. : 4
	Effective Date: 8-August-2005
<p style="text-align: center;">INTRODUCTION</p> <p>THE DNA IQ™ SYSTEM</p> <p>Many procedures are available for the purification of DNA from evidentiary samples containing biological material. Commonly used methods are phenol/chloroform extraction, Chelex® extraction, and QIAGEN® column affinity purification. Phenol/chloroform extraction of samples requires the use of hazardous chemicals and may result in some loss of material. While rapid, the Chelex® extraction method does not remove amplification inhibitors and the DNA can degrade over time. Purification on silica matrices such as the QIAGEN® column has some advantages since the method does not require organic compounds, is easy to use and usually removes PCR inhibitors. However, it can be costly.</p> <p>Promega Corporation's DNA IQ™ System is also an affinity based purification system that utilizes the affinity of DNA for silica in a manner similar to the QIAGEN® column method. However, unlike the QIAGEN® columns where the silica is utilized as a filter, the DNA IQ™ System uses a silica coated paramagnetic resin. Since a paramagnetic resin is utilized with the system, no centrifugation steps are required and instead, cell debris, buffers and washes are removed when the paramagnetic resin/cell lysate suspension is placed into a magnetic stand or if used in conjunction with the BIOMEK®2000 robot, placed onto the magnetic plate. The paramagnetic resin is pulled to the side of the tube in proximity to the magnet and the buffer can be removed without disrupting the paramagnetic resin pellet. The paramagnetic particles used with the DNA IQ™ System are designed not to clump when coated with DNA, which is a problem with many other silica particles. One benefit to the use of the paramagnetic resin is its easy adaptation to "hands off" robotics since no centrifugation or filtration steps are required.</p> <p>The DNA IQ™ System is designed to efficiently purify small quantities of DNA. The paramagnetic resin saturates at approximately 100 ng of bound DNA. Excess DNA is removed during the DNA purification process. The DNA IQ™ System becomes more efficient with samples containing less than 50 ng of DNA, which is frequently the case with evidentiary forensic samples.</p> <p>THE BIOMEK®2000 AUTOMATION WORKSTATION</p> <p>The BIOMEK®2000 Automation Workstation is manufactured by Beckman Instruments, Inc. The robot is used extensively in biotechnology research and private industry laboratories, as well as in pharmaceutical laboratories. The particular BIOMEK®2000 Automation Workstation pictured in Figure 1 has been modified by Promega Corporation for the purpose of utilizing the DNA IQ™ System for DNA extraction.</p> <p>The BIOMEK®2000 Automation Workstation is a fairly simple robot. It picks up tips, 8 at a time, which constitutes a column on a 96 well plate. It also has a gripper tool for picking up and moving plates around. A shaking platform for mixing samples has been attached to the right hand side of the robot. Mounted on the shaking platform, is a thermal exchange unit coupled with a heat transfer plate, for the elution of the DNA from the DNA IQ™ paramagnetic resin. The thermal exchange unit is heated via its attachment with tubing to a circulating water bath. Specially adapted aerosol resistant tips are used with the BIOMEK®2000 Automation Workstation. The tip boxes have enclosed wells so that the tips can be re-used wherever possible, but the used tips will not contaminate any neighboring tips.</p> <p>The BIOMEK®2000 Automation Workstation has a limited number of sensors. For example, it is unable to detect a clogged tip. Therefore samples with large fragments of undigested material, such as what is frequently found in a tissue digest, should be centrifuged for several minutes to pellet the debris, prior to loading the lysate into a 96 well plate for robotic DNA extraction.</p>	

INTRODUCTION	Page vi
BIOMEK®2000 AUTOMATION WORKSTATION PROCEDURES MANUAL - FORENSIC BIOLOGY SECTION PROCEDURE MANUAL, SECTION IV	Issue No. : 4
	Effective Date: 8-August-2005

All of the labware holders (gray clamps) and tip box holders (black clamps) can be easily removed and repositioned on the deck. Likewise the tool stands (used to hold the Gripper, MP200, P200L, MP20, and P20 tools) lifts out and can be positioned elsewhere on the deck if another method requires it.

Software methods have been written by scientists at Promega Corporation for use with the DNA IQ™ System on the BIOMEK® 2000 Automation Workstation. The methods are employed for the extraction of mixed stains, tissue, bloodstains and buccal cells described in this manual. The methods are the same except for the number of samples extracted (24, 40, 56 and 80 samples). The methods involve the use of deep well plates for the initial loading of cell samples, automated resin addition and dispensation of purified DNA into strip tubes. The strip tubes can be cut apart such that samples may be returned to the individual examiners.

Figure 1. The BioMek®2000 Automation Workstation with modifications for DNA extraction using the DNAIQ™ System.



- 1 = Microtube rack for holding strip tubes for the purified DNA (position B1)
- 2 = Beckman deepwell 96 well plate for loading cell samples (position B2)
- 3 = Reservoir holder for buffers and strip tubes (position B3)
- 4 = Deep well plate for waste disposal (position B4)
- 5 = Magnetic plate (position B5)
- 6 = Shaking platform with labware holders
- 7 = Thermal exchange unit (position A6)
- 8 = Deck
- 9 = Gripper tool (position A3)
- 10 = MP20 pipetting tool (position A3)
- 11 = MP200 pipetting tool (attached to robot arm)
- 12 = Water bath for heating thermal exchange unit

INTRODUCTION	Page vii
BIOMEK®2000 AUTOMATION WORKSTATION PROCEDURES MANUAL - FORENSIC BIOLOGY SECTION PROCEDURE MANUAL, SECTION IV	Issue No. : 4
	Effective Date: 8-August-2005
<p data-bbox="245 268 1097 302">THE ALUQUANT® HUMAN DNA QUANTITATION SYSTEM</p> <p data-bbox="245 338 1520 506">The AluQuant® Human DNA Quantitation System utilizes two enzymatic steps in order to produce ATP in a quantity proportional to the amount of human DNA present in a sample. The ATP is then utilized by the luciferase enzyme when it oxidizes its chemical substrate, luciferin, which then produces light. The measurement of the light produced provides information regarding the amount of human DNA in a sample when compared with the light produced from DNA standards of known concentration.</p> <p data-bbox="245 541 1539 848">Initially, the DNA samples are denatured in a NaOH solution, followed by addition of HCl to bring the pH back to a more neutral value. Afterwards, the AluQuant® Probe, containing DNA sequences which are human specific, is added to the DNA samples in a master mix which also contains the AluQuant® Enzyme Solution. The AluQuant® Enzyme Solution contains the READase™ Polymerase and READase™ Kinase. The READase™ Polymerase depolymerizes the double stranded DNA and removes NTPs from the 3' terminus of the probe by the addition of pyrophosphate to the 3'-terminal base. The READase™ Kinase uses the released NTPs to perform terminal phosphate transfers onto ADP, creating ATP. It is this ATP product that is used by the luciferase enzyme in the presence of the luciferase reagent to create light. The light is measured and quantified in a luminometer.</p> <p data-bbox="245 884 1520 1052">Human genomic DNA is provided as a quantitation standard from which a dilution series is generated. Once the luciferase signal from the DNA standards and the DNA samples are measured using a luminometer, the data, in relative light units (RLU), are imported into an Excel macro. The Excel macro, the AluQuant® Calculator, generates a standard curve from the DNA standards and extrapolates the DNA concentrations of the samples using the standard curve.</p> <p data-bbox="1468 1121 1544 1150" style="text-align: right;">◆END</p>	